A diverse family of inositol 5-phosphatases playing a role in growth and development in Dictyostelium discoideum

Loovers, HM; Veenstra, K; Snippe, H; Pesesse, [No Value]; Erneux, C; van Haastert, PJM; Loovers, Harriët M.; Pesesse, Xavier

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Appendix A: Amino acids sequences of Dd5P1, Dd5P2, Dd5P3 and Dd5P4.

Amino acids represented in figure 2 are underlined and shown in blue.

Dd5P1

MVLRGRAGSMTPQVNFNKLSGSGNIEERNNNQSSSQSQSSPSPSQSPSLQQNQLNSSNGM
NNKVKNLLDLENYYKSIKKNITENQFEFSIKKKEKNRTLLVNLKDKSLTIFKLSKRGNKK
NIKKYLISEIEKITKDKKDNKELIVFRRSKKKEMKLYFLNTERRECFYELIWMTWNNKIM
PPTPLSGEEDVEEKAQYEKISI**FISTWNMG**DAPPPINEGLLNSWIPKSKPY**DLYVIGVQE**
CEYTPLKSKHKQTQLNQPQQQPQPQPQQAQAQQQPQQQNQSQQQYSECQEDWFGTLSEHL
GHDYYKVESTSLVKMRIIIFAKKQHYYKINYIEKASIGTGIGNMYGNKGGTCISLSWWET
SFCFMSSHFAAHQEKIEQRNSNYKDIIKGIKIGNKELDILNQYNYV**FWMGDLNYRI**GGGL
FREEVLMHIKSKNIRKLLQHDQLNQQRNQEKVFIGFKEEPVG**FLPTYKTL**RGVENQYTEE
KQ**RIPSWCDRIL**HKKLPYSHDIICLEYNSANRITT**SDHVPV**YGVYESYVRMPCSPNSSSC
SNVKKQICQILFNDLRAECLDMVDNEERAPDAYIVFNSSAFIATEITTPHAYKNRNPIWG
DLPPITPIIERQNFLETQHLFLTFYHDDKSIGHAAVPLGIGFQKEPFAFKTRITRNGLPA
GTLFGSIHIVYNKFLIQN

Dd5P2

MGKRKTGNTCHNIDKKYPSPPSVLISLNLDQLSMGPNHSAAIVNNECLMWGEVMGRKHTT
LFKIVWKRRVVSVSCGFNHTLLLTSEYKIFSMGFNQTGQLGIENVVDSAVPLPVDTLSKF
NVIKIKAGNNVSAAITEAGDLYCWGSSAYGQLGNGKMNAKLHSFTPTTILNPLIPDAEKD
DPIYQNLQYTNICFGAQHTIAISTTGAIFSWGSNQYGQLGLGHCNAKLVPVPIHLDNSFE
RRAGHINSLGEIVTTDFTAKTIHRIVVDIACGNFHSALLTLEGDLFLWGSNQYGQLGQGD
SLDQSVPKTVKGSLEFVKINRMALGNNHTILLTKEQDVFTFGSNEFYQLGHGSIGGKNRL
YFPTQIQKPKVMASDIFAAGDCSAYFVLDDSPVHLNLLGQHQLLNGAGGNGGGGGSSNNN
NNLLNNYNNGLMGSTNKHLSI**FVGTWNCN**AKRTQNLANWILTNSFAP**DIIVLGLQEI**VNM
KAGAIVKATAADKQNNKENAYHPWKHDIEQTLSLSSGGRYVKVMNKVLVGLMILVYVKEE
HAPYISEVSGAVVPCGMMGKIGNKGGLGVRFTLYKTGICFVNSHLAAGPSHEKMERRTQD
YKKIQMMTFENHLSMLDHECL**IWFGDLNYRI**DLPYSETKALIEQKNWQKLQAHDQLNNER
KAGKAFIGFKEELTN**FAPSYKYD**IGVNQYDTSEKN**RTPSWCDRII**YRGESLKQISYQRHE
LME**SDHRPI**SAIFLLETKEYIHAESSKAWSKFKHKDHANRSDSPDPFFSKIGSAAHHFSP
VELKSSSSSINSTNSSVDSLVGSISSSNTTNSTNTTTDSINIINTSTKRNLPNLPAPPLP
PKSNAVKKSLLNIHQHHSHSGSGSIGGSSSGNPFVVGSMPTFSPHFMYSNSPTSSDTSTN
SGVTLNDSPMIRSILKNNAINKRLSFSAEDIHSVKVLSNHRKIKLN?NNNNNNNNNNNNN
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EEEEEEDEEDDEDFIDSESDSDVGGYCSSMPTLNSLGDSGACHLEDLPLNFLKNREINYG
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VEHHDEENCSNDIFYIEPFTIVDQYTNNNNNNNNNNNNNNNNNNDNNNDNNNDNNNDND
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KQSPSS?S?QSLKNESEFICVSPSSPSRVLFNPIFDKSFFEQKHRVVNTDIVLGSH?RSS
PPVISPIGEFNSTTNSTTNSTT?STTNSNSNINTKTNAINNNNKSDRELDKLKTKSGGSS
FINIKSANSSRNNSMIYHSIENRHNNYNSDDSEDDGISYNINSDSDSDSDSHSDTNENSN
INIEPKTIEPKIIEPTTSIATTTEPTTSTEIETNTTVTTSTDPKLPTEKEGSSL

Dd5P3

MADNTFRIFITTQSFIIKRDSLHSKALVINRRDPSKIDIIQPDQIDQSLRSERSVYCVLG
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GIDDDGNVANFCESEQILSIESYGVLAFLQIRGSVPVFWNQSSPQMSDLKIKMSNLSKIG
KISKKKITIARNTQATTPAFQLHLKEQTKKYGNIVIVNLLSKLKSGECDLVNAYEEQIRI
LRSPQVFYNHFDLNEQTKGNRMDPLDSLINYIDNQSISGGGGTTGGVSEKIVGYFFQNSN
GQIISKQNGIIRTNCKDCLDRTNIVQSRVSWVLFESQLRKLGLFRGHDSIGSYPRVSQTL
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LDLLLGLHKSMVVNNSNNGNGGNGGNGSGHDDNIRKQVQDRVKEYSKKEWRNV**FVGTYNV**
GGVHSSYFDLVQWLRPDGANCPTP**DFYVLGLQEV**VELTAGQILATDSSIGKQWEDAIERA
LPKVSPNVKYIKLQSSQLVGLLMAIYVREDAIHYFREVQIQNVKCGMNGLAGNKGGIGVR
LLFSDTSFTFVTAHFAAGQSNVEDRISDFREIDSTLAFGRQGQFKVSDSDYS**FWLGDFNF**
RIDLPDEEIRRCVYDQQFPKLYNYDQLRKCMEAGRVFNGYREETIA**FPPTYKYD**LNSTQY
DTSQKQ**RSPAWTDRIV**WSNKVHHDLRQLYYHRQEILA**SDHRPV**SSYFQLEVTKIDKDKER
TLRQELYEQKSSQFITTSQSTNNLINTTNQNGKDGANLITKEFEQLTLIKQSASTPNLQK
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TGSGGGRKISSSPSSSFNNTNSIGSSSSPSGAHSPIFDDQFDPRKGRSSPVPQSFNENNN
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LQPIHQSNLNPNSNYNQQLQQPPPQPINHDLQLIDSWGNSSNGYQSREPPPLQPIPQSVY
QSQPPPLQPIPQSVYQPTNFALQQPLQPMMSPPPLLYSNQKAVYNIPVPSPYNQQQQQPQ
QPYQQPYQQPYQQPYQQNLYNQPPPLNNSYNNYNNQNNNNNNQNNNNTYNNPYLGSILD

Dd5P4

MGDIQNTDNIESNIDNNNNNNVSLESSSSSQNNNTNTTTTTTTTSVDNLQVGVLSISDQS
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NKLKERESEFTEKRGMSI**FLGTWNVN**GKKPSESLDPWLKDPSMSLQP**DIYAIGFQEL**DLT
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AVGIMGMMGNKGGVAIRFSFYNTTICILNSHLNAHMDNVLRRNQDMKDISKNIKFINESS
TDHSTINIFDHDQL**FWIGDLNYRI**PLPDNEVKEKIKKKDFYNLFLVDQLNQQMKAGAVFE
GFQEPPIS**FAPTYKYD**AGTEEYDSSEKK**RTPAWCDRIL**WKTHKKAENVGILSYKRAELIS
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PISKQLIFENIGQVIARFQFIPKLDETILCKPWLKISPLAGMMIPKEKVTIDLTIYVDNL
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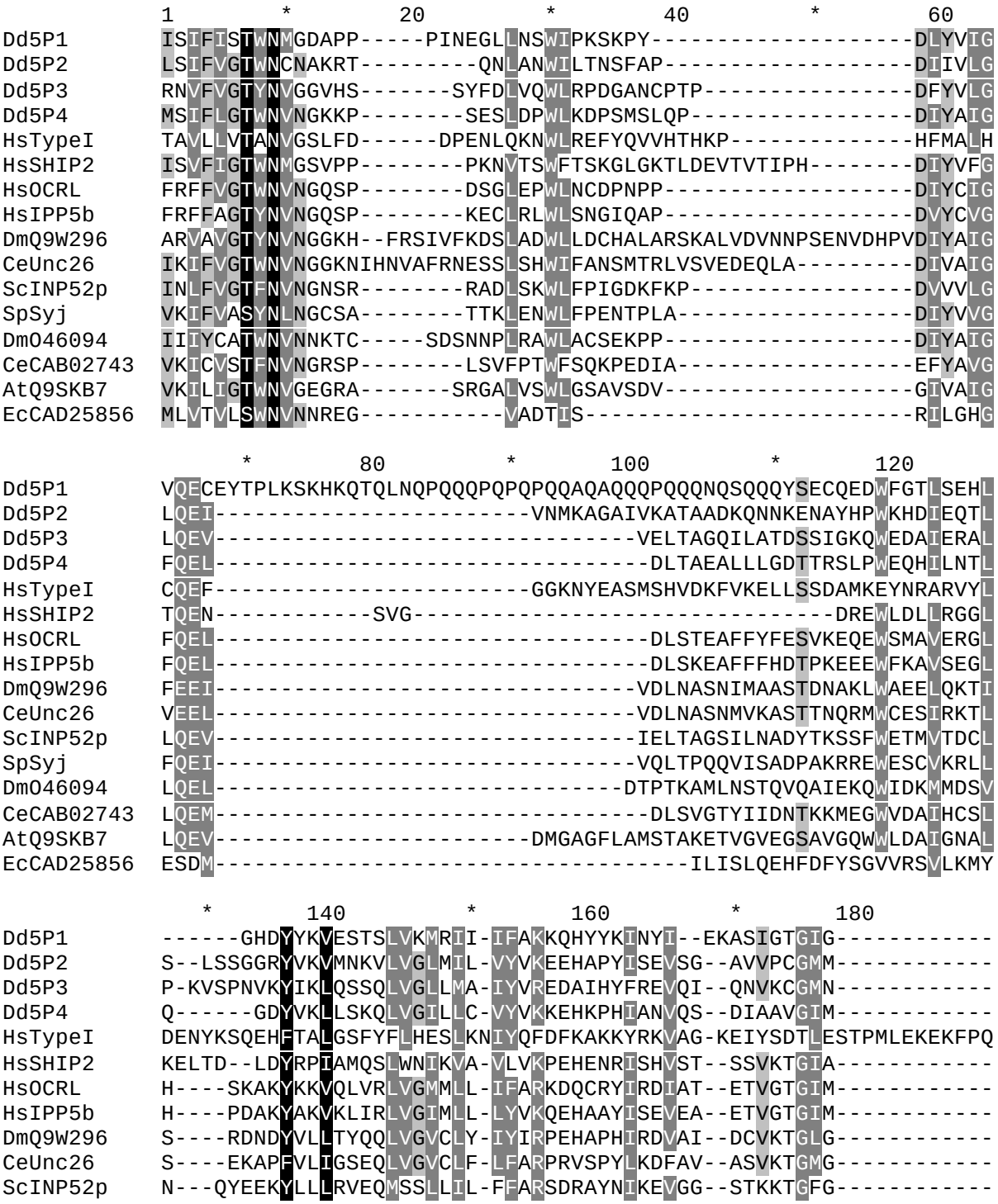
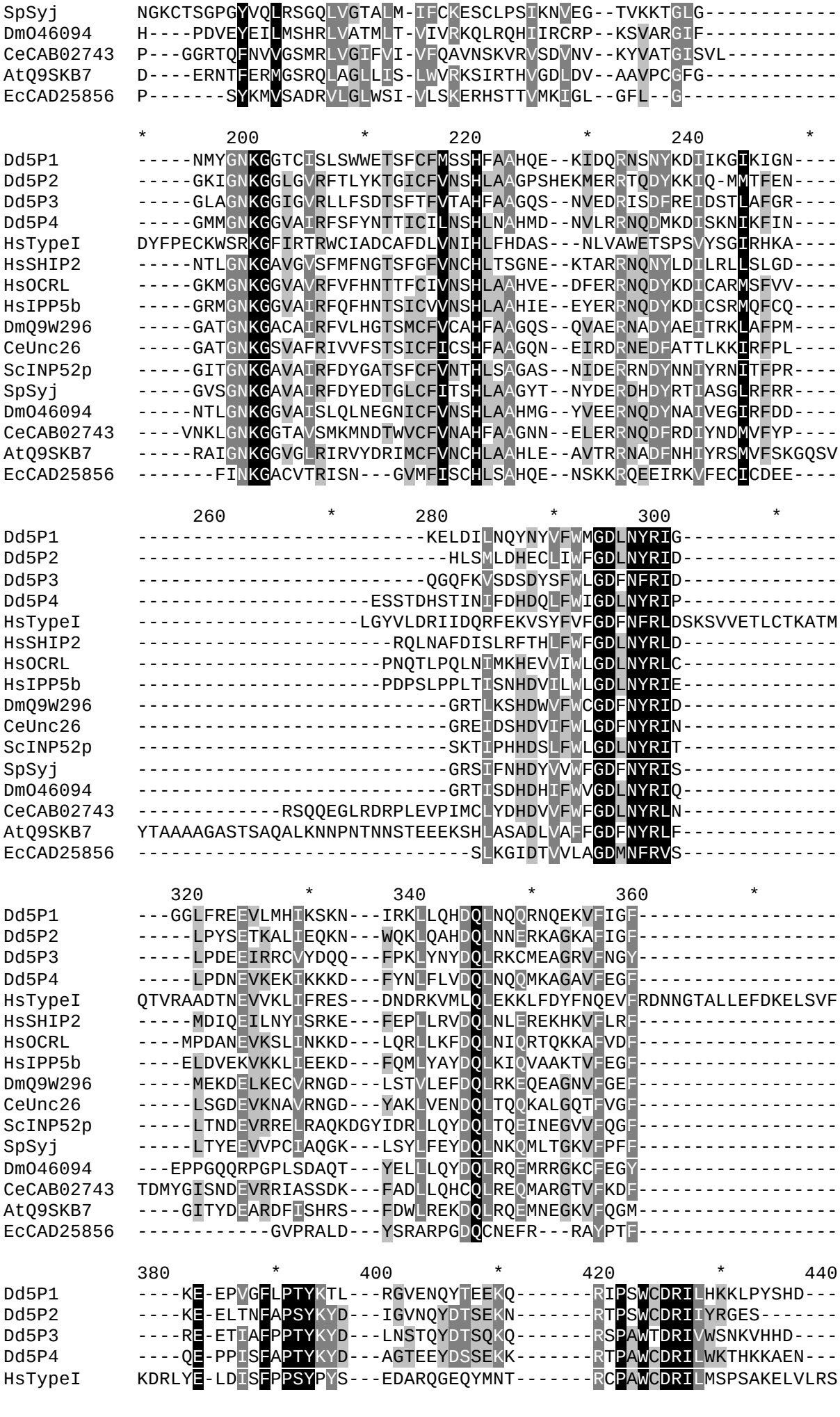
RNNLPIPPEQKKLSIPKELWRIIDYIYYNGLKEEGLFIKSGVTKEMELIRDCLDTAEPFS
SISFSIHSMAETLIRFLESLVEPVIPFNMYQQALDASSSPLSCKTLVSHLPSVNYNVFFY
LISFLIETLSNQKENDLKPDQLAIIFSTVLLRPSPQSQLSQFPPDATTVKKKADLILHFL
ISKDLIN

Appendix B: **Amino acids sequence alignment of the catalytic domains of inositol 5-phosphatases.** First two letters indicate the species: At, *Arabidopsis thaliana*; Ce, *Caenorhabditis elegans*; Dd, *Dictyostelium discoideum*; Dm, *Drosophila melanogaster*; Ec, *Encephalitozoon cuniculi*; Hs, *Homo sapiens*; Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*. Genebank entries: AtQ9SKB7, Q9SKB7; CeUnc26, Q9GT42; CeCAB02743 (Synaptojanin-like), CAB02743; Dd5P1, pending; Dd5P2, pending; Dd5P3, pending; Dd5P4, pending; DmO46094 (OCRL-like), O46094; DmQ9W296 (Synaptojanin-like), Q9W296; EcCAD25856, CAD25856; HsTypeI, Q14642; HsSHIP2, O15357; HsOCRL, AAA59964; HsINPP5b, P32019; ScINP52p, NP_014293; SpSyj, 1I9Y_A. Conserved regions are shown in black (100% conserved), gray with white letters (80-100% conserved) or gray with black letters (60-80% conserved).

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CeCAB02743 P---GGRTQFNVVGSMRLVGIFVI-VFQAVNSKVRVSDVNV--KYVATGISVL----------
AtQ9SKB7 D----ERNTFERMGSRQLAGLLIS-LWVRKSIRTHVGDLDV--AAVPCGFG----------
EcCAD25856 P-------SYKMVSADRVLGLWSI-VLSKERHSTTVMKIGL--GFL--G----------
200 220 240
Dd5P1 -----NMYGNKGGTCISLSWWETSFCFMSSHFAAHQE--KIDQRNSNYKDIIKGIKIGN----
Dd5P2 -----GKIGNKGGLGVRFTLYKTGICFVNSHLAAGPSHEKMERRTQDYKKIQ-MMTFEN----
Dd5P3 -----GLAGNKGGIGVRLLFSDTSFTFVTAHFAAGQS--NVEDRISDFREIDSTLAFGR----
Dd5P4 -----GMMGNKGGVAIRFSFYNTTICILNSHLNAHMD--NVLRRNQDMKDISKNIKFIN----
HsTypeI DYFPECKWSRKGFIRTRWCIADCAFDLVNIHLFHDAS---NLVAWETSPSVYSGIRHKA----
HsSHIP2 -----NTLGNKGAVGVSFMFNGTSFGFVNCHLTSGNE--KTARRNQNYLDILRLLSLGD----
HsOCRL -----GKMGNKGGVAVRFVFHNTTFCIVNSHLAAHVE--DFERRNQDYKDICARMSFVV----
HsIPP5b -----GRMGNKGGVAIRFQFHNTSICVVNSHLAAHIE--EYERRNQDYKDICSRMQFCQ----
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CeUnc26 -----GATGNKGSVAFRIVVFSTSICFICSHFAAGQN--EIRDRNEDFATTLKKIRFPL----
ScINP52p -----GITGNKGAVAIRFDYGATSFCFVNTHLSAGAS--NIDERRNDYNNIYRNITFPR----
SpSyj -----GVSGNKGAVAIRFDYEDTGLCFITSHLAAGYT--NYDERDHDYRTIASGLRFRR----
DmO46094 -----NTLGNKGGVAISLQLNEGNICFVNSHLAAHMG--YVEERNQDYNAIVEGIRFDD----
CeCAB02743 ----VNKLGNKGGTAVSMKMNDTWVCFVNAHFAAGNN--ELERRNQDFRDIYNDMVFYP----
AtQ9SKB7 -----RAIGNKGGVGLRIRVYDRIMCFVNCHLAAHLE--AVTRRNADFNHIYRSMVFSKGQSV
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260 280 300
Dd5P1 -------------------------KELDILNQYNYVFWMGDLNYRIG--------------
Dd5P2 ----------------------------HLSMLDHECLIWFGDLNYRID--------------
Dd5P3 -------------------------QGQFKVSDSDYSFWLGDFNFRID--------------
Dd5P4 ----------------------ESSTDHSTINIFDHDQLFWIGDLNYRIP--------------
HsTypeI --------------------LGYVLDRIIDQRFEKVSYFVFGDFNFRLDSKSVVETLCTKATM
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HsOCRL ----------------------PNQTLPQLNIMKHEVVIWLGDLNYRLC--------------
HsIPP5b ----------------------PDPSLPPLTISNHDVILWLGDLNYRIE--------------
DmQ9W296 --------------------------GRTLKSHDWVFWCGDFNYRID--------------
CeUnc26 --------------------------GREIDSHDVIFWLGDFNYRIN--------------
ScINP52p --------------------------SKTIPHHDSLFWLGDLNYRIT--------------
SpSyj --------------------------GRSIFNHDYVVWFGDFNYRIS--------------
DmO46094 --------------------------GRTISDHDHIFWVGDLNYRIQ--------------
CeCAB02743 ------------RSQQEGLRDRPLEVPIMCLYDHDVVFWFGDLNYRLN--------------
AtQ9SKB7 YTAAAAGASTSAQALKNNPNTNNSTEEEKSHLASADLVAFFGDFNYRLF--------------
EcCAD25856 ----------------------------SLKGIDTVVLAGDMNFRVS--------------
320 340 360
Dd5P1 ---GGLFREEVLMHIKSKN---IRKLLQHDQLNQQRNQEKVFIGF------------------
Dd5P2 -----LPYSETKALIEQKN---WQKLQAHDQLNNERKAGKAFIGF------------------
Dd5P3 -----LPDEEIRRCVYDQQ---FPKLYNYDQLRKCMEAGRVFNGY------------------
Dd5P4 -----LPDNEVKEKIKKKD---FYNLFLVDQLNQQMKAGAVFEGF------------------
HsTypeI QTVRAADTNEVVKLIFRES---DNDRKVMLQLEKKLFDYFNQEVFRDNNGTALLEFDKELSVF
HsSHIP2 -----MDIQEILNYISRKE---FEPLLRVDQLNLEREKHKVFLRF------------------
HsOCRL ----MPDANEVKSLINKKD---LQRLLKFDQLNIQRTQKKAFVDF------------------
HsIPP5b ----ELDVEKVKKLIEEKD---FQMLYAYDQLKIQVAAKTVFEGF------------------
DmQ9W296 -----MEKDELKECVRNGD---LSTVLEFDQLRKEQEAGNVFGEF------------------
CeUnc26 -----LSGDEVKNAVRNGD---YAKLVENDQLTQQKALGQTFVGF------------------
ScINP52p -----LTNDEVRRELRAQKDGYIDRLLQYDQLTQEINEGVVFQGF------------------
SpSyj -----LTYEEVVPCIAQGK---LSYLFEYDQLNKQMLTGKVFPFF------------------
DmO46094 ---EPPGQQRPGPLSDAQT---YELLLQYDQLRQEMRRGKCFEGY------------------
CeCAB02743 TDMYGISNDEVRRIASSDK---FADLLQHCQLREQMARGTVFKDF------------------
AtQ9SKB7 ----GITYDEARDFISHRS---FDWLREKDQLRQEMNEGKVFQGM------------------
EcCAD25856 ------------GVPRALD---YSRARPGDQCNEFR---RAYPTF------------------
380 400 420 440
Dd5P1 ----KE-EPVGFLPTYKTL---RGVENQYTEEKQ-------RIPSWCDRILHKKLPYSHD---
Dd5P2 ----KE-ELTNFAPSYKYD---IGVNQYDTSEKN-------RTPSWCDRIIYRGES-------
Dd5P3 ----RE-ETIAFPPTYKYD---LNSTQYDTSQKQ-------RSPAWTDRIVWSNKVHHD----
Dd5P4 ----QE-PPISFAPTYKYD---AGTEEYDSSEKK-------RTPAWCDRILWKTHKKAEN---
HsTypeI KDRLYE-LDISFPPSYPYS---EDARQGEQYMNT-------RCPAWCDRILMSPSAKELVLRS

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HsSHIP2      ----SE-EEISFPPTYRYE---RGSRDTYAWHKQKPTGVRTNVPSWCDRILWKSYPETH----
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HsIPP5b      ----TE-GELTFQPTYKYD---TGSDDWDTSEKC-------RAPAWCDRILWKGKN-------
DmQ9W296     ----LE-GEITFDPTYKYD---LFSDDYDTSEKQ-------RAPAWTDRVLWRRRKALAEGDF
CeUnc26      ----NE-GQLTFAPTYKYD---TFSDDYDTSEKC-------RAPAWTDRILWKDQRKKGK---
ScINP52p     ----KE-PTLQFRPTYKYD---YGTDNYDTSEKA-------RTPSWTDRIIYKGEN-------
SpSyj        ----SE-LPITFPPTYKFD---IGTDIYDTSDKH-------RVPAWTDRILYRGE--------
DmO46094     ----TE-GEIKFRPTYKYD---PGTDNYDSSEKQ-------RAPAYCDRVLWKGTR-------
CeCAB02743   ----EEPSTLPFRPTYKYD---CGTNTWDTSEKG-------RVPAWTDRILTFKKYPQVGLES
AtQ9SKB7     ----RE-ALITFPPTYKFEKNKPGLGGYDSGEKK-------RIPAWCDRVIYRDNQSISYTEC
EcCAD25856   ----LE-EVIRFGPTYKY------ITGTDELCRK-------RHPSWCDRVFVSSSCT------

                     *       460         *       480
Dd5P1        ----------IICLEYNS-ANRITTSDHVPVYGVYESYVRMPCSPNSS
Dd5P2        ----------LKQISYQ--RHELMESDHRPISAIFLLETKEYIHAESS
Dd5P3        ----------LRQLYYH--RQEILASDHRPVSSYFQLEVTKIDKDKER
Dd5P4        ----------VGILSYK--RAELISSDHRPVSASFVIKIKVVIPDSKN
HsTypeI      E-------SEEKVVTYDHIGPNVCMGDHKPVFLAFRIMPGAGKPHAHV
HsSHIP2      ----------IICNSYGC-TDDIVTSDHSPVFGTFEVGVTSQFISKKG
HsOCRL       ----------VNQLNYRS-HMELKTSDHKPVSALFHIGVKVVDERRYR
HsIPP5b      ----------ITQLSYQS-HMALKTSDHKPVSSVFDIGVRVVNDELYR
DmQ9W296     AASA---WNPGKLIHYG--RSELKQSDHRPVIAIIDAEIMEIDQQRRR
CeUnc26      ----------TQLLSYD--RSELKTSDHRPVGAVFKVETFKVGGRKCV
ScINP52p     ----------LHPLAYS--DAPLKISDHKPVYAAYRANVKFVDEKEKL
SpSyj        ----------LVPHSYQ--SVPLYYSDHRPIYATYEANIVKVDREKKK
DmO46094     ----------IEQLAYNS-IMEIRQSDHKPVYAVFQVKVKTRDEVKYK
CeCAB02743   I---------RPMVSIDT----ITISDHKPVRAMFNLKVKKINESGAN
AtQ9SKB7     SLKCPVV---SSTIMYEA-CMDVTESDHKPVRCKLHANIAHTDKSVRR
EcCAD25856   ----------CRFNTYSS-IHSVKISDHKPVVCIFEVDGKRTNRIAIP
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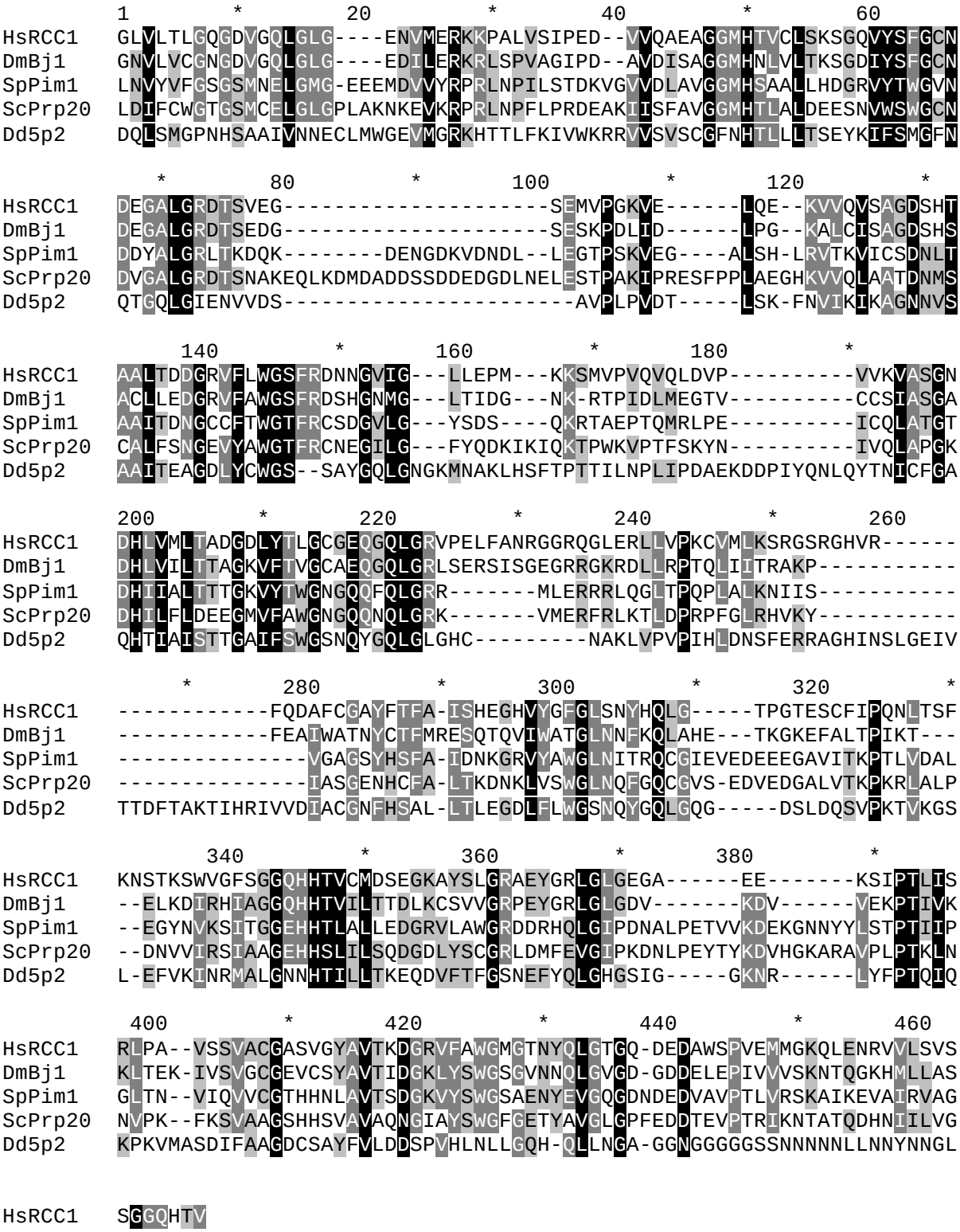
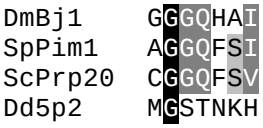
Appendix C: **Amino acids sequence alignment of RCC1 domains.** First two letters indicate the species: Dd, *Dictyostelium discoideum*; Dm, *Drosophila melanogaster*; Hs, *Homo sapiens*; Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*. Genebank entries: Dd5P2, pending; DmBj1, P25171; HsRCC1, CAA31182; ScPrp20, P21827; SpPim1, P28745. Conserved regions are shown in black (100% conserved), gray with white letters (80-100% conserved) or gray with black letters (60-80% conserved).

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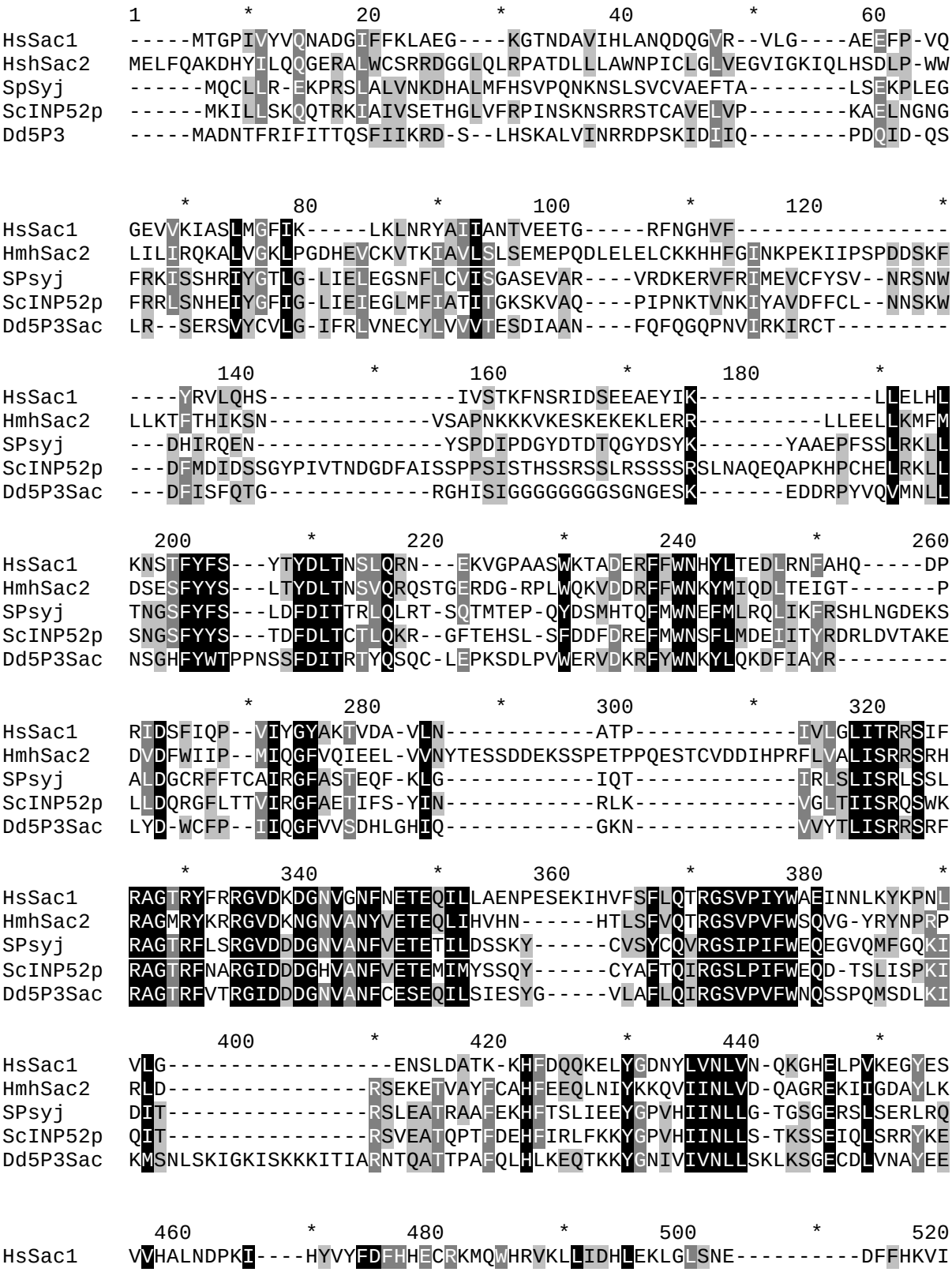
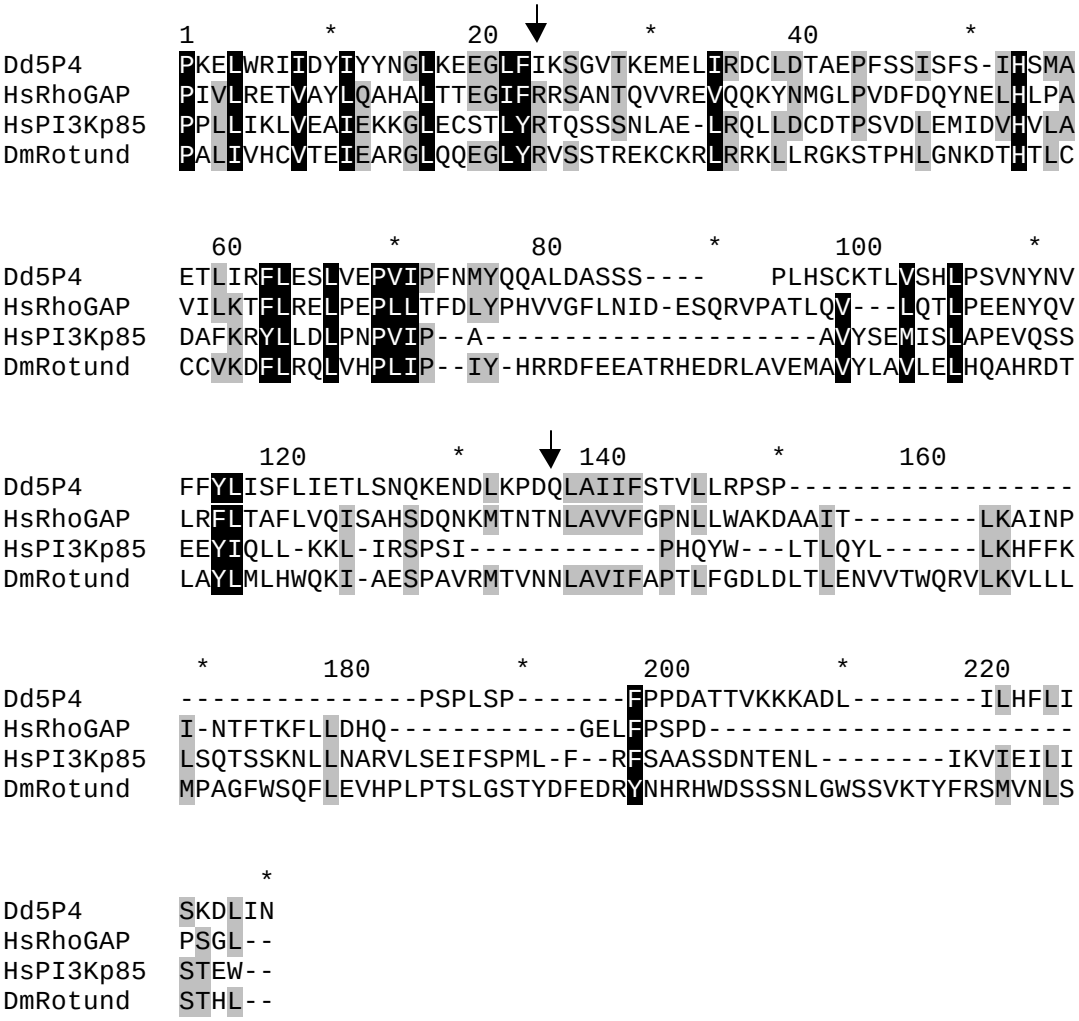
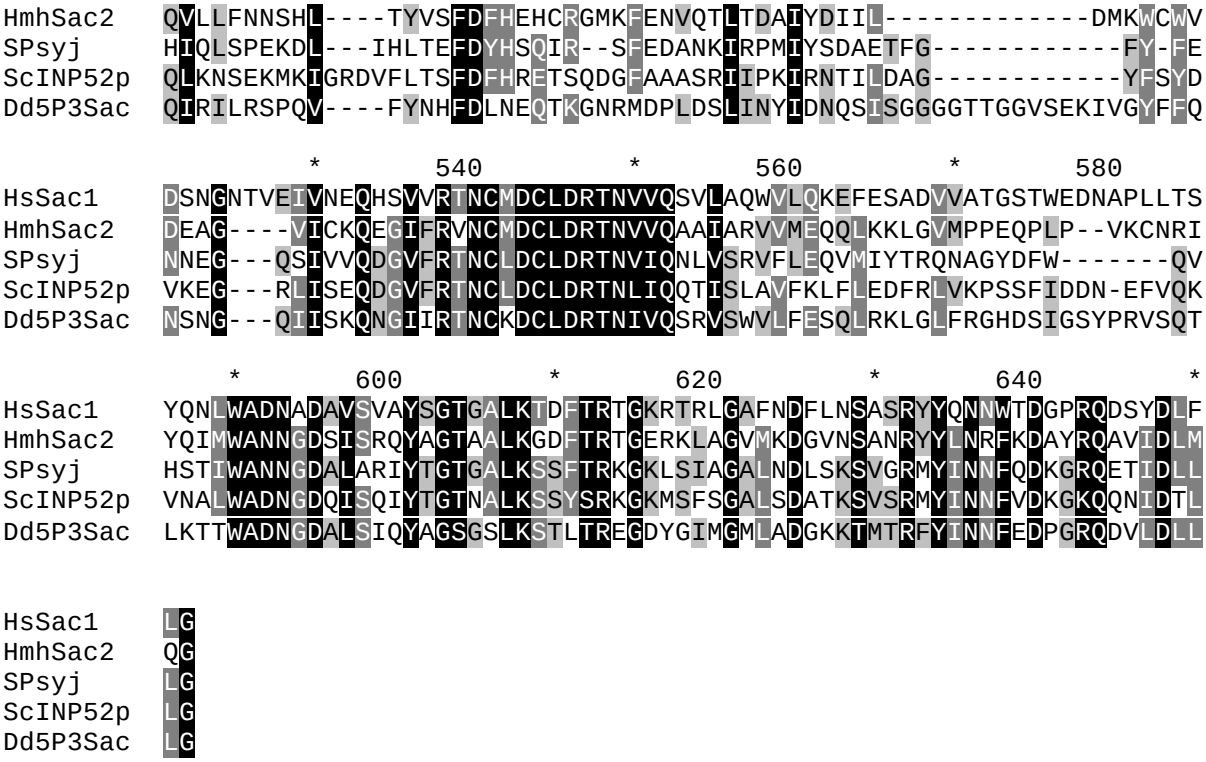
SpPim1 AGGQFSI
ScPrp20 CGGQFSV
Dd5p2 MGSTNKH

Appendix D: **Amino acids sequence alignment of Sac1 domains.** First two letters indicate the species: Dd, *Dictyostelium discoideum*; Hs, *Homo sapiens*; Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*. Genebank entries: Dd5P3, pending; HsSac1, NP_054735; HshSac2, NP_055752; ScINP52p, NP_014293; SpSyj, 1I9Y_A. Conserved regions are shown in black (100% conserved), gray with white letters (80-100% conserved) or gray with black letters (60-80% conserved).



Appendix E: **Amino acids sequence alignment of RhoGAP domains.** First two letters indicate the species: Dd, *Dictyostelium discoideum*; Dm, *Drosophila melanogaster*; Hs, *Homo sapiens.* Genebank entries: Dd5P4, pending; HsRhoGAP, Q07960; HsPI3Kp85, P27986; DmRotund, AAL59599. Conserved regions are shown in black (100% conserved) or gray (80-100% conserved). Arrows indicate the position of $Arg85_{p50RhoGAP}$ and $Asn194_{p50RhoGAP.}$

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ScINP52p QLKNSEKMKIGRDVFLTSFDFHRETSQDGFAAASRIIPKIRNTILDAG----------YFSYD
Dd5P3Sac QIRILRSPQV----FYNHFDLNEQTKGNRMDPLDSLINYIDNQSISGGGGTTGGVSEKIVGYFFQ
* 540 * 560 * 580
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SPsyj NNEG---QSIVVQDGVFRTNCLDCLDRTNVIQNLVSRVFLEQVMIYTRQNAGYDFW-------QV
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Dd5P3Sac NSNG---QIISKQNGIIRTNCKDCLDRTNIVQSRVSWVLFESQLRKLGLFRGHDSIGSYPRVSQT
* 600 * 620 * 640 *
HsSac1 YQNLWADNADAVSVAYSGTGALKTDFTRTGKRTRLGAFNDFLNSASRYYQNNWTDGPRQDSYDLF
HmhSac2 YQIMWANNGDSISRQYAGTAALKGDFTRTGERKLAGVMKDGVNSANRYYLNRFKDAYRQAVIDLM
SPsyj HSTIWANNGDALARIYTGTGALKSSFTRKGKLSIAGALNDLSKSVGRMYINNFQDKGRQETIDLL
ScINP52p VNALWADNGDQISQIYTGTNALKSSYSRKGKMSFSGALSDATKSVSRMYINNFVDKGKQQNIDTL
Dd5P3Sac LKTTWADNGDALSIQYAGSGSLKSTLTREGDYGIMGMLADGKKTMTRFYINNFEDPGRQDVLDLL
HsSac1 LG
HmhSac2 QG
SPsyj LG
ScINP52p LG
Dd5P3Sac LG

Appendix E: **Primers mentioned in Materials and Methods**

1OES2	AATTAGGATCCAAAAATGTTAATAGTTTTTAGAAGGAG
1OER1	TTTATAAGATCTTTACTCCTCATTGTCAACCATATC
2OES1	TTATAGGATCCAAAAATGAATGGATTAATGGGTAG
2OER1	TTATAAGATCTTTAAACTGGAGAGAAATGATGAG
4OES1	AATTAGGATCCAAAAATGGGTGATATTCAAAATACAG
4OER1	ATATAAGATCTTTAATCTGGCATACTACTATTCTC
5OES2	TTATACTCGAGAAAAATGAATGGTAGTGGTCATGATG
5OER2	ATATTCTGCAGTTAAAGATTATTGGTACTTTGAGAGG
5P2S1	TGATGAATTCAGTAAAAGCAACAGCAGCC
5P2R1	ACAATCTAGAGCTTTCGTTTCAGAGTAAGG
5P5S1	ATTGCTGCAGAATCATCACAATTGGTAGG
5P5R1	TGTTACTAGTAACCATTGAACACTCTACG
5P4S1	CATCTCTAGAGCTGGAGCAGTATTTGAAGG
5P4R1	CACAACTAGTTTGTTTCATCTAGCTTTGG
6BIr	TTACTCTTTATATGCATTAGCACC
6BIf	ATACATCGATGAGAAGCAACG
OE4GAPs1	AATTAGGATCCAAAAATGTCAACAAATGAATCAATGGATG